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AN APPROACH TO THE STUDY OF ENVIRONMENTAL MICROBIAL AEROSOLS

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ABSTRACT

Environmental microbial aerosols are formed by processes such as wastewater treatment. The significance of these aerosols to human populations is dependent upon the concentration and type of microorganisms contained therein and upon the probability of susceptible population exposure to the infectious organisms. An approach to the study of environmental microbial aerosols to meet research objectives requires consideration of the environmental conditions during the study as well as assurance of quality throughout the sample collection and assay procedures.

KEYWORDS

Microbial aerosols, wastewater treatment, air sampling, sampling sensitivity, air sampling instrumentation.

INTRODUCTION

Microbial aerosols are emitted by wastewater treatment processes into the surrounding air (Hickey and Reist, 1975). Activated sludge treatment, for example, generates small bubbles by diffused air aeration that adsorb and concentrate suspended bacteria and viruses as they rise through the sewage depth in the aeration tank to the surface boundary. At this boundary, a surface film containing microorganisms is disrupted as these rising bubbles burst, releasing tiny aerosol droplets containing the bubble-adsorbed, as well as the surface film-associated microorganisms (Woodcock, 1955; Blanchard and Syzdek, 1970; Baylor, Peters and Baylor, 1977). Spray irrigation equipment produces large quantities of respirable aerosols whose number increases as a function of windspeed and length of time exposed to the wind (Raynor and Hayes, 1976). During treatment by trickling filtration, impacting sewage onto rocks within the trickling filter unit causes a splashing effect, resulting in sewage aerosol formation (Adams and Spendlove, 1970). The nearly instantaneous evaporation which may occur as these droplets become suspended in air leaves dried residues referred to as droplet nuclei (Riley, 1974) that are subject to downwind dispersion. The survival and dispersion of the organisms that may be associated with these droplet nuclei is affected by organism characteristics and environmental factors such as relative humidity, temperature, irradiation, wind velocity, atmospheric stability, and atmospheric pollutants.

Tor 1104 K. F. FANNIN

The purpose of this presentation is to outline factors that require consideration in an approach to the study of the microbial aerosols in the ambient environment.

DEFINITION OF OBJECTIVES

Field studies of microbial aerosols, initiated as specific research investigations or as monitoring programs intending to answer questions with health, political, economic, or regulatory implications, are dependent upon the clarity of proposed objectives for their ultimate success. These objectives are the basis of study site selection, experimental design, methods selection, and monitoring strategies. The study site selection is also dependent upon factors such as physical accessibility, cooperation of local governmental officials, and density and location of a potentially exposed population. Since the study objectives may require aerosol concentration discrimination on the basis of time, location, season, particle size, or atmospheric stability, an experimental design may require utilization of several sampling instruments at numerous locations at specified time intervals. Determination of both sampling frequency and number of sampling locations should be made in an effort to obtain statistically reliable data. A compromise between either sampling frequency or number of sampling locations may, however, be required when resource limitations are a factor.

DETERMINATION OF REQUIRED SAMPLING SENSITIVITY

Guidelines regarding required microbial aerosol detection sensitivities have not been defined. As a result, air sampling volumes collected during specific field studies are often based upon the constraints of existing sampling methodologies and may be below the sensitivities required for detection of low-level aerosols. The significance of nonpositive observations is dependent upon the proportion of the total air volume being sampled, which is very small in open air, and upon the efficiency of the collection and assay systems. With this in mind, negative observations must be interpreted in view of the detection sensitivities attained. Prior to initiating a field sampling program, the investigator should determine a level of acceptable sampling sensitivity at which negative observations will be interpreted as inconsequential.

A level of acceptable risk of exposure, and resultant sampling sensitivity, to microbial aerosols has not yet been determined but may be based upon either individual or population exposure. That is, either the exposure of an individual over a limited time period (Schaub, Glennon and Bausum, 1978) or that of a susceptible population of a certain size and density may be emphasized in such risk determinations. With the latter approach, introduction of an infection into a population via droplet nuclei and subsequent transmission by effective contact between susceptible and infected individuals should be considered (Riley and O'Grady, 1961).

While health effects can be assessed by epidemiological methods, determination of risks of exposure requires the application of reliable methodologies capable of detecting airborne microbial contaminants at acceptable sensitivity levels. Sample collection from open air is complicated by a high dilution factor for dispersed particulates. Consequently, attaining the sensitivity required for detecting source-related infectious microbial aerosols at the threshold of acceptable risk may require sampling at relatively high air flow rates for long time periods. Extensive instrumentation, based on widely varying concepts, have been developed for detecting viable microbial aerosols. Each device has unique characteristics which often result in differences in collection efficiencies for aerosols in certain size ranges, sampling capacities, and operational capabilities

that make comparisons of data collected with different samplers or under differing conditions very difficult. Although an attempt was made to recognize certain low-volume samplers as standards for studies in aerobiology (Brachman and co-workers, 1964), the need for samplers of greater sensitivity for field studies has resulted in the development of non-standard large-volume air samplers based upon different collection concepts.

VIABLE MICROBIAL AEROSOL SAMPLING CONCEPTS AND INSTRUMENTS

The principle aerosol collection concepts which have been used in aerobiology are sedimentation, filtration, impingement, electrostatic precipitation, cyclone scrubbing, and impaction. Typical examples of the application of each concept for viable microbial aerosol collection are presented in Table 1.

TABLE 1 Selected Viable Microbial Aerosol Sampling Concepts and Instruments

Type of Sampler	Collection Surface	Relative Air Sampling Capacity	Comments
Sedimentation			
Open Petri Dish	Solid	Low	Simple, inexpensive, non-quantitative, favor particles with high settling velocity
Filtration			
Membrane Filter	Solid	Low	Simple, relatively inexpensive, viability losses due to desiccation of fragile organisms
Electrostatic Precipitation			
Litton Model M; LEAP	Liquid	High	Complex, expensive, sterilization difficult
Centrifugal Separation			
Cyclone Scrubber	Liquid	High	Simple, relatively inexpensive, autoclavable
Impingement			
All-Glass Impinger	Liquid	Low	Simple, inexpensive, dependable conventional usage
Multistaged Impinger	Liquid	Low	Simple, relatively inexpensive, size classification
Multislit Impinger	Liquid	High	Complex, expensive, undependable, sterilization difficult
Impaction			
Slit Sampler	Solid	Low	Simple, time discrimination of aerosols
Multistaged Impactor	Solid	Low	Simple, relatively expensive, particle size discrimination, dependable, conventional usage

Sedimentation

Settling plates offer a simple and inexpensive method of microbial aerosol collection. The aerosol particles are permitted to settle on a nutritional agar-based medium contained within petri dishes. Following incubation, colonies are counted and the general level of contamination for a defined time period is assessed. Large particles of greater density have a higher settling velocity than smaller particles of lower density and are, consequently, collected more efficiently. Since smaller particles are more likely to achieve deep lung penetration than are larger ones (Hatch, 1961), the value of microbial aerosol collection by sedimentation in the assessment of the risk of respiratory infection is limited by its failure to detect more minute aerosols.

Filtration

Filtration is another simple and relatively inexpensive commercially available means of efficient removal of microorganisms from an airstream. Stable microorganisms such as Bacillis subtilis var. niger spores, for example, can be efficiently recovered by filtration (Buchanan and co-workers, 1972), while the recovery of viable vegetative Serratia marcescens has been shown to be as low as 0.1% to 2% under certain conditions (Goetz, 1953). Viability losses due to desiccation of fragile organisms makes use of filter samplers impractical for long-term or large volume sampling applications.

Impingement

As used in this presentation, impingement refers to striking into rather than onto a collection medium. Liquid impinger samplers are used to determine the number of viable microorganisms in aerosols, rather than the number of viable aerosol particles (May and Harper, 1957). The all-glass impinger (AGI) is a simple, inexpensive, easily sterilized device designed for isokinetic sampling of dynamic aerosols (Tyler and Shipe, 1959) at 0.0125 m^3 per minute by high speed impingement into a collecting medium. The AGI-30 (having a jet-to-base distance of 30 mm) was recommended as a standard sampler by a committee of aerobiologists (Brachman and co-workers, 1964).

Impinger sampling fluid is under low pressure and tends to evaporate quickly and freeze in cool dry air (Anderson and Cox, 1967). Changes in fluid pH, volume, and osmotic pressure can occur during liquid impingement necessitating sampling time restrictions (Hyslop, 1972). Relatively low air sampling rates and short permissible sampling durations limit the sensitivity of impinger samplers. To increase impinger sampling capacity and, hence, sensitivity, a large volume multi-slit impinger (MSI) was developed (Buchanan and co-workers, 1968). The MSI, which collects aerosol particles at an air sampling rate of about 1 m^3 per minute by inertial impaction into a liquid film-coated rotating disc, has been used in field studies of wastewater-generated microbial aerosols (Fannin and co-workers, 1976). In addition to difficulties in field dependability reported by these workers, the MSI does not lend itself to conventional sterilization procedures prior to sample collection.

Electrostatic Precipitation

The concept of aerosol collection by electrostatic precipitation onto a wetted surface has been employed in the development of large volume air samplers, with air sampling rate capacities ranging from about 1 m^3 to 10 m^3 per minute.

Both the Litton Model M (LVAS) (Decker and co-workers, 1969) and the Environmental Research Corporation LEAP (Lundgren and Graf, 1964) electrostatic precipitator large volume air samplers have been used in field studies of wastewater-generated microbial aerosols (Fannin and co-workers, 1977; Johnson and co-workers, 1978). In addition to being difficult to sterilize by conventional methods, problems with sampling fluid evaporation and quantitation exist with the large volume air samplers (Gerone and co-workers, 1966).

Cyclone Scrubbing

The viable microbial aerosol cyclone scrubber samplers collect airborne particles by tangential impingement into a continuous fluid film created as a mist is impacted onto the sampler wall. The fluid film travels spirally to a reservoir where it is collected by slight suction. Such devices, constructed of glass, plastic, or stainless steel have air sampling rates up to about 0.95 m^3 per minute and fluid flow rates from 1 to 4 ml per minute (Decker and co-workers, 1969; Errington and Powell, 1969; Buchanan and co-workers, 1972; White and co-workers, 1975). The scrubbing liquid, supplied either by peristalic or screw-driven syringe pumps is subject to water loss due to evaporation which may require replacement.

Cyclone scrubber samplers lend themselves to sterilization by conventional processes and have been used in field studies for recovery of wastewater-generated aerosols (Teltsch and Katzenelson, 1978). In the field, cyclone scrubbers are relatively easy to operate, compared to other existing large volume air samplers, but may require continuous monitoring and fluid adjustments to minimize fluid losses by slippage during sample collection.

Impaction

After acceleration to sufficient inertia to leave an airstream, aerosol particles can be impacted onto a collecting medium surface. Acceleration through slits or jets of critical size and distance from the impaction surface is widely used for collection of microbial aerosols onto agar media. Examples of such devices include slit and multistaged impactors. The most common forms of both instruments sample at a rate of about 0.028 m^3 per minute. With the slit sampler, airborne particles are collected onto a slowly rotating agar surface for time discrimination of microbial aerosol concentrations (Bourdillon, Lidwell, and Thomas, 1941), while the multistaged impactor, such as that developed by Andersen (1958), permits aerodynamic particle sizing by impaction onto the surface of staged agar-containing plates at increasing velocities. Wide acceptance of this multistaged impactor led to its recommendation as a standard sampler for collection of airborne microorganism-containing particles (Brachman and co-workers, 1964).

The sampling capacity of both the slit sampler and multistaged impactor is limited by their relatively low air sampling rates and by observations of decreased collected organism viability during prolonged sampling periods (Kuehne and Decker, 1957; Andersson, Bergstrom and Bucht, 1973). The sampling period in both samplers can be extended without affecting the viability of collected organisms by applying an evaporation retardant to the agar surface (May, 1969). To further increase the air sampling capacity of agar impactors, larger volume instruments have been developed. The Cassella multi-slit impactor, designed for sampling up to 0.7 m^3 per minute, and the Pagoda multistaged impactor, reported capable of sampling particles into distinct size ranges at rates up to 1 m^3 per minute were described by May (1973). A major limitation of agar impactor samplers in general is that they are best suited for enumeration of microorganism-containing particles rather than for total bacteria or viruses, for which a liquid medium would be better suited.

Tor 1108

K. F. FANNIN

SELECTION OF AIR SAMPLING INSTRUMENTATION

Depending upon the objectives of the particular study, several types of samplers may be required for simultaneous sampling. If, for example, high sensitivity as well as particle size and time discrimination are required, such devices as large volume air samplers, multistaged impactors, and slit samplers may be employed. In practice, each sampler has unique advantages and disadvantages which should be considered prior to sampler selection or data interpretation. Prior to selecting specific sampling methodologies, consideration should be given to criteria such as: 1) collection efficiency; 2) sampling sensitivity; 3) reliability; 4) ease of sterilization; 5) maintenance of collected sample viability; 6) ease of sample assay; 7) ease of operation; 8) remote operation capability; 9) particle size discrimination; and 10) cost. Using these criteria, a sampler can be evaluated to determine its applicability for a specific field study. Conventional aerobiological sampler evalutations, performed by relative rather than absolute methods, determine microbial aerosol collection efficiencies as a percentage of that observed in a reference sampler. The sensitivity of a particular sampler is directly related to its collection efficiency and to other operational parameters. The sensitivity of various samplers under specified conditions can be compared by determining a value which could be referred to as a coefficient of sensitivity defined as:

$$CS = \frac{CE \cdot R \cdot D}{V} \qquad (1)$$

where:

CS = coefficient of sensitivity

CE = collection efficiency (percent of reference sampler)

R = air sampling rate (m^3/min)

D = sampling duration capability (min)

V = final volume of collection medium (ml)

The CS value increases with collection efficiency, air sampling rate, sampling duration capability, and with reductions in the final collection fluid volume required for assay. One way to reduce this final collection fluid volume is by recirculation during the sampling process. Depending upon the organisms collected, however, such a practice could result in some viability losses which would, in turn, reduce the estimated viable microorganism collection efficiency.

The reliability, ease of sterilization, ease of operation and remote operation capacity vary substantially among existing viable microbial aerosol samplers. Sampler reliability, which is related to the dependability and robustness of individual components, is of critical importance in the field when replacement parts and repair services are not available. Samplers designed with heat-sensitive components integrally associated with aerosol collection or sample moving systems do not lend themselves to sterilization by conventional steam autoclaving procedures and may require the selection and evaluation of other means of sterilization. Independent sampler operation during field studies requires varying degrees of operator training. Such devices as slit samplers, for example, are relatively simple to operate compared to electrostatic precipitator large volume air samplers and, consequently, require substantially less intensive personnel training prior to field use. Although, in practice, most viable microbial samplers require some

attention in the field, those with greater remote operation capacity offer more versatility for field use than those which require constant attention.

The kind of sampler selected depends upon the study objectives and the assay procedures. If, for example, an objective is to assess risks of respiratory infections, then a sampler classifying the microbial aerosol according to particle size should be employed. Although agar-based samples are easy to assay for certain kinds of bacteria or fungi, samplers employing a liquid collecting medium permit versatility by enabling both sample dilutions and greater assay technique versatility. Bacteria and viruses, for example, may be assayed from the same liquid medium sample. During sample collection, transport, and assay, an environment compatible with the survival of specific kinds of organisms should be employed. Generally, this requires continuous hydration of the organisms in a collection medium at a temperature compatible with organism survival, but not growth.

The cost of viable microbial aerosol samplers vary substantially. Glass impinger samplers are, for example, inexpensive while the cost of certain large volume electostatic precipitators is relatively high. When resource limitations are a factor, relative costs must be considered in the sampler selection process.

SELECTION OF SAMPLING MEDIA

Sampling medium selection is based on individual sampling program requirements and is not yet standardized for general application. Microbial aerosols may be collected in any medium that is compatible with study objectives and that facilitates efficient collection, promotes viability maintenance, and lends itself to assay in the system selected. Knowledge of the physical and chemical characteristics of the microorganisms under study will help to determine the chemical medium composition that will enhance viability maintenance during the collection process.

Although the use of a fluid sampling medium, followed by inoculation into a susceptible host or cell system, generally is most satisfactory for general use in virus aerosol studies (Noble, 1967), a solid medium may be more suitable for other microbial aerosol applications. If the study objectives include determination of the number of microorganism-containing particles rather than the number of microorganisms in the aerosol, for example, it may be necessary to use a solid rather than a fluid sampling medium. The physical state of the required medium together with the estimated detection sensitivity needed, will help determine the kind of sampler most suitable for the study.

FIELD SAMPLING PROCEDURES

All sample and data analyses, interpretations, and conclusions are dependent upon the reliability and validity of the sample collection process. Along with the problems of selecting instrumentation and personnel, the procedures employed in the field must be carefully planned. If resources are limited, a smaller number of sensitive samples may be of greater value to project objectives than a large number of insensitive samples. The representativeness of a particular sample will be affected by air sampling velocities, sampler inlet orientation, and wind speed and direction fluctuations. Under normal ambient conditions, isokinetic sampling is very difficult and impractical to achieve (May, 1945). In addition to sample collection difficulties, other logistical problems such as those of electrical supply requirements, frequent sampler breakdowns in the field, maintaining sampler sterility, field communication, transportation of sampling equipment at field site, and transport of collected samples to the laboratory for assay have been noted

(Schaub, Glennon and Bausum, 1978) and must be considered. Sample handling during the sampling process and transport to the laboratory for assay must be done to minimize the possibility of contamination and microbial die-off or growth.

LABORATORY ANALYSIS AND EVALUATION

When available, standard procedures should be employed for the assay of specific microbiological parameters (Standard Methods for the Evaluation of Water and Wastewater, 14th ed., 1975; Bordner, Winter and Scarpino, 1978). Precise methods for sample analyses are affected by sampling fluid composition and required detection sensitivities. Samples collected on an agar-based medium, for example, may only require incubation prior to enumeration whereas those collected in a liquid medium may, depending on the test organism, require simple dilution and plating, filtration, or elaborate processing procedures prior to assay.

Microbial aerosol concentrations are expressed as detectable assay units per m^3 of air. Enumeration can be based upon direct counts or multiple tube dilution methods, such as the most probable number (mpn) procedures, for estimation of the number of detectable assay units per sample volume. Application of multiple tube dilution procedures has been shown, in some instances, to be more sensitive than direct enumeration methods for detecting certain microorganisms (Hoff and Jakubowski, 1966). Selection of specific enumeration procedures, however, may be based upon specific project objectives, assay methods, and sensitivity requirements.

Microbial aerosol concentrations from fluid medium samplers can be determined by:

$$C = \frac{N \cdot V}{A \cdot R \cdot D} \qquad (2)$$

where:

C = concentration of detectable microbial units per m^3 air (e.g., cfu/m^3)

N = number of detectable microbial units (e.g., cfu, mpnpfu)

A = portion of sample assayed (ml)

V = final volume of collection fluid (ml)

R = air sampling rate (m^3/min)

D = sampling duration (min)

For agar-based medium samplers, A and V may be omitted unless only a portion of the total sample is to be counted. In such a case, the volume of air per cm^2 agar surface area would be used in concentration calculations. If the sampler collection efficiency is known, then the C value should be appropriately corrected.

QUALITY ASSURANCE

Methods for detecting viable microbial aerosols are not yet standardized. Consequently, there may be considerable variation between the results among investigators using different sampling or assay procedures. The efficiency of viable microbial air samplers depends upon a number of operational parameters, including air sampling and fluid flow rates, wind velocity and direction fluctuations, aerosol particle size and density variations, as well as the overall efficiency of

the sampler itself. The efficiency of specific samplers may be determined relative to a reference sampler under experimental conditions for aerosols having known characteristics. Such determinations, however, are relative and not absolute. Under varying conditions, such as those encountered in the field, true sampling efficiencies cannot easily be ascertained.

Prior to use and periodically throughout the project, each sampler should be calibrated to determine air flow rates using a precalibrated mass flow meter or anemometer. All samplers should be numbered and each collected sample identified with a particular instrument. Controls for verification of field sampler sterility and laboratory assay procedures should be employed where appropriate. In addition to quality assurance procedures required for the specific project, standard laboratory and assay procedures should be adhered to where applicable.

DATA ANALYSES AND INTERPRETATION

Microbial aerosol concentration data may be collected under a wide range of meteorological conditions; each having effects on microbial survival, dispersion of heterogeneously sized aerosols, and aerosol sampling efficiencies. Existing environmental conditions that affect the data may be either known or unknown to the investigator. For example, composite coliform bacteria-containing particle data, obtained by different investigators under varying conditions and shown in Table 2, suggest the existence of unknown or unreported sources at a location downwind from some trickling filter processes. When additional sources exist,

TABLE 2 Coliform Bacteria-Containing Particles[a] Downwind From Three Wastewater Treatment/Utilization Processes[b]

Distance Downwind (m)	Spray Irrigation	Trickling Filter	Activated Sludge
<20	430 (2)[c]	250 (37)	74 (51)
20-49[d]	380 (12)	210 (12)	82 (8)
50-99	110 (2)	130 (9)	--
100-199	14 (3)	330 (13)	--
200-299	3 (1)	250 (4)	--
300-399	3 (4)	--	--
≥400	1 (4)	120 (9)	--

[a] Determined on Endo or EMB agar.

[b] Source: Fair and Wells, 1938; Nepolitano and Rowe, 1966; Adams and Spendlove, 1970; Goff and co-workers, 1973; Fannin, 1976; Katzenelson and Teltsch, 1976; Teltsch and Katzenelson, 1978; Carnow and co-workers, 1979.

[c] Parentheses encloses number of samples.

[d] Distance defined as "away from tank or filter" placed in this category.

expected downwind concentration-distance relationships, as observed with the spray irrigation-related data, may be eliminated. The occurrence of such sources upwind from the study site can render uncertain value to upwind control samples, further confounding data interpretation.

In contrast to the findings of Nepolitano and Rowe (1966), whose data are also included in Table 2, the observations of these several studies show higher coliform bacteria-containing particle concentrations around trickling filter than activated sludge processes. Within 49 m of the three wastewater treatment processes presented, the highest concentrations were observed around spray irrigation processes. Consistent with these relative observations, recent reports using existing methodologies have shown detectable animal viruses around spray irrigation (Teltsch and Katzenelson, 1978; Moore, Sagik and Sorber, 1979; Teltsch and co-workers, 1980), but not around activated sludge or trickling filter processes (Fannin and co-workers, 1977). Data interpretation must take into account the effects of existing environmental conditions and the limitations of the methodologies employed to obtain the information.

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